FEDERAL EXPERIMENT STATION IN PUERTO RICO
of the
UNITED STATES DEPARTMENT OF AGRICULTURE
MAYAGUEZ, PUERTO RICO

REPORT OF THE FEDERAL EXPERIMENT STATION IN PUERTO RICO 1953

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FEDERAL EXPERIMENT STATION IN PUERTO RICO

MAYAGUEZ, P. R.

Administered by the Office of Experiment Stations, Agricultural Research Administration, United States Department of Agriculture

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[1] In cooperation with the Government of Puerto Rico.

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REPORT OF THE FEDERAL EXPERIMENT STATION IN PUERTO RICO, 1953

CONTENTS

The research program of the Federal Experiment Station in Puerto Rico has continued its investigations of problems and crops of strategic and economic importance to Puerto Rico and the continental United States.

Closer cooperation with other bureaus of the Department has resulted in greater utilization of the station as an outpost of the Department on problems of national interest that can be attacked to the best advantage in the Tropics. Studies on the cultural requirements of tropical plants like Strophanthus and Dioscorea, possible sources of cortisone, and studies on the biosynthesis of rubber in Hevea are examples of some of the cooperative investigations of importance that were undertaken.

The outstanding research contributions during the year were the isolation and identification of hiptagenic acid, a toxic compound found in trailing indigo. A fundamental study on phosphorous metabolism gave evidence on the mode of action of 2,4-D in plants. Studies on the persistence and movement of CMU in the plant and soil also yielded important basic information in the weed-control project. Important advances were made in the forage-improvement program, in studies of the pollination of Hevea, and in the control of vanilla root rot.

The following pages contain summary accounts of the research results obtained during the year.

PERSONNEL

There were no new appointments or resignations in the Federal staff during the year. The Director was appointed Head of the Division of Tropical Agriculture of the Office of Experiment Stations with responsibility for administering the Federal Experiment Station in Puerto Rico and the new Virgin Islands Agricultural Research and Extension Program. A considerable amount of time was spent on the organization of the Virgin Islands program, which was placed under the jurisdiction of the United States Department of Agriculture as of July 1, 1952.

Dr. L. G. Saunders, professor of entomology, on sabbatical leave from the University of Saskatchewan, Canada, spent January to March at the station conducting investigations on the heleid midges, with particular emphasis on the larval stages of those responsible for the pollination of Hevea.

Jean García-Rivera, chemist employed with funds appropriated by the Government of Puerto Rico, resigned on August 15, 1952, to join the Bureau of Standards in Washington, D. C. Jesús Arvelo was appointed to fill the vacancy on March 24, 1953.

COOPERATION WITH OTHER GOVERNMENT AGENCIES

Funds amounting to $45,000 were appropriated by the Government of Puerto Rico for the Federal Experiment Station to carry out cooperative experimental work on agricultural problems of particular interest to Puerto Rico, including investigations on vanilla, spices, weed control, essential oils, and bamboo.

The Experiment Station of the University of Puerto Rico and the Federal Experiment Station continue to maintain close relations. The cooperative papaya and forage-improvement projects were continued. Through conferences of the Directors and staff members, the two stations maintained a well coordinated program.

The Extension Service of the University of Puerto Rico assisted in the distribution of plant material to farmers, and in the dissemination of technical information developed by the station.

The Forest Service continued to make available to the station land at Toro Negro, Maricao, and Guanica, for the testing of various tropical plants.

The Puerto Rico Industrial Development Company cooperated with the station in the distribution of cured bamboo culms for industrial purposes.

A number of projects were carried out in cooperation with other bureaus and agencies of the Department. Office, laboratory space, and other station facilities were provided for Dr. D. M. Eny, biochemist, employed by the Division of Rubber Plant Investigations of the Bureau of Plant Industry, Soils, and Agricultural Engineering, to study basic problems in the production of rubber latex. Also, a cooperative project was continued with the Rubber Division to study problems of pollination and propagation in *Hevea brasiliensis*. Dr. H. E. Warmke, of the station staff, was sent to Central America for a 6-week period to study Hevea pollination. A project to test rust resistance was carried on cooperatively with the Division of Cereal Crops and Diseases. Investigations on the improvement of sweet-

potatoes and the introduction of varieties resistant to fusarium wilt were carried out in cooperation with the Vegetable Crops Division of the same bureau. Experimental trials with various species of *Strophanthus* and *Dioscorea* were continued in cooperation with the Division of Plant Exploration and Introduction, BPISAE.

Office space was provided for the Soil Conservation Service and for the plant quarantine inspector of the Insular Plant Quarantine Service.

Close relations were maintained with the Office of Foreign Agricultural Relations of the Department in the exchange of technical information and plant material. The cooperative bamboo experiments initiated 4 years ago in collaboration with Dr. F. A. McClure, Field Service Consultant on Bamboo for the Foreign Agricultural Service, were continued.

Station facilities and personnel were utilized to train Point IV appointees from a number of tropical countries.

Individuals and various companies have extended cooperation in many ways. Through the cooperation of agencies and individuals scattered throughout the world, many additions were made to our large collection of tropical plants.

IMPROVEMENT OF PHYSICAL PLANT

The areas in which the offices, laboratories, staff houses, greenhouses, and principal experimental plots are located were completely enclosed with cyclone fence. This installation has reduced pilfering and other forms of vandalism in connection with experimental work. A fence 12 feet high made of railroad rails and heavy wire fencing was constructed along the boundary of the Paris field. Considerable difficulty has been experienced in this area in the past by the adjoining neighbors stealing, pasturing their animals, and throwing garbage and other refuse on the station grounds. The present fence should eliminate this problem.

The permanent irrigation system in the main experimental area was lengthened and new portable aluminum tubing and accessories were installed. Practically the entire area can now be watered as needed.

A low swampy area between the college and the station has been drained, filled, and the land reclaimed for experimental and plant introduction use.

A small laboratory for conducting studies with radio isotope C^{14} was constructed adjacent to the rubber biochemistry laboratory and a chemical hood equipped for isotope work was installed in the biochemistry laboratory.

The new warehouse and garage building was put in final condition for use by completing the installation of electrical fixtures, the construction of workbenches and shelves, painting the woodwork, and by mounting a hydraulic grease rack for car maintenance.

INSECTICIDAL-CROP INVESTIGATIONS

DERRIS BREEDING. E. Cabanillas and H. E. Warmke.

More than one thousand additional crosses were made during the year among four varieties of *Derris elliptica* (Wall.) Benth. (Changi, Sarawak Creeping, St. Croix, and Sumatran). From these, only six viable seeds were produced. Spraying the flowering branches at

weekly intervals with solutions containing 5 p. p. m. of seven growth-promoting substances failed to improve seed set or retention. The chemicals used were para-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, chlorophenoxypropionic acid, 2,4-dichlorophenoxypropionic acid, 2,4,5-trichlorophenoxypropionic acid, butyl 2,4,5-trichlorophenoxyacetate, and naphthaleneacetic acid.

Absorption and Translocation Studies with Derris. C. Pagán and M. P. Morris.

A series of tests were conducted to determine the ability of young bean plants to absorb and translocate the toxic components of derris root and to determine the stability of the deposit on the leaves.

Several forms of rotenone-bearing materials including the latex, powdered derris root, and pure rotenone were applied to leaves of young bean plants. In one experiment the bean plants were grown in soil watered with a suspension of the powdered roots. The plant parts were analyzed periodically for rotenone, using both chemical and bioassay methods.

The data obtained showed that the toxicity of deposits of fresh derris root latex, powdered derris roots, and rotenone on young bean seedlings disappeared almost completely within 10 days; and rotenone was not absorbed and translocated in amounts that could be detected by a sensitive bioassay method or by chemical tests, and therefore is of no value as a systemic insecticide.

Mamey Toxicity. J. García-Rivera and M. P. Morris.

The fruit of the mamey tree has been eaten by man in tropical America for many years. This fruit is processed and sold in the form of jellies and is available in season at the markets throughout tropical America. Since several highly toxic compounds have been isolated from the seeds of mamey fruit, feeding tests were carried out to determine if these toxic compounds occurred in the edible portion of the fruit.

The edibility studies of fresh mamey were carried out by feeding extracts of the edible portion to guinea pigs. Each of a group of six, 1-pound adult guinea pigs was fed daily the extract (300 mg.) of a single mamey. All died within 4 days. Each of another group of six, 1-pound, adult animals was fed the extract of one-half of a mamey fruit. Four animals died within 7 days. One died on the ninth day, and one was alive after 12 days of treatment. Each of a third group of animals was fed the extract of one-fourth of a mamey fruit. Three died within 8 days, and three were alive after 12 days of treatment, at which time all animals, including three controls, were returned to normal rations.

These tests indicate that the edible portion of mamey contains substances highly toxic to guinea pigs and further studies showed that these substances were toxic also to dogs and cats.

DRUG-CROP INVESTIGATIONS

Strophanthus Cultural Requirements. H. F. Winters and F. Rodríguez-Colón.

Practically nothing is known about the cultural requirements of Strophanthus, a vine from tropical West Africa, the seed of which contains a steroid that can be converted into cortisone. A series of

cultural experiments was started in cooperation with the Division of Plant Exploration and Introduction of the Bureau of Plant Industry, Soils, and Agricultural Engineering, to study the effect of mulching and fertilization on growth and flowering of *Strophanthus* sp. The data obtained to date show no consistent differences between mulching treatments but the fertilized plants are superior to those not fertilized in number and length of branches and in diameter of the main stem at ground level. After one year in the field the plants showed no indication of flowering, but there was a tendency to become dormant during the dry season as evidenced by yellowing and loss of foliage.

DIOSCOREA TRIALS. W. C. Kennard and F. Llavat-Cristy.

Dioscorea tubers and plants representing 52 selections as possible cortisone sources were received in the autumn of 1951 from the Division of Plant Exploration and Introduction, Bureau of Plant Industry, Soils, and Agricultural Engineering. The selections were collected in Central and South America and in Africa. Two of the collections have been identified as *Dioscorea macrostachya* Benth., 46 have been listed as *Dioscorea* species, and the remaining 4 have not been identified. These selections were planted in individual pots in the greenhouse and later were transplanted to the field where they showed great variation in vigor and growth habit. One selection did not produce any vine growth, whereas others produced long vigorous vines with many leaves. Tuber yields per plant varied from 2 grams to 9,255 grams. The greatest yield for any one selection was 19,380 grams, the lowest 7 grams. A sample of the selections which produced a sufficient quantity of tubers was sent to the Bureau of Plant Industry, Soils, and Agricultural Engineering for assay.

FOOD-CROP INVESTIGATIONS

SWEETPOTATO BREEDING. E. Cabanillas and H. E. Warmke.

Sixteen additional sweetpotato seeds were harvested from crosses made at Mayaguez during the 1952–53 season. The season was most unfavorable for sweetpotato breeding. The flowering season was short, lasting only during the months of December and January, and blossoms were never abundant. Many capsules started to enlarge after crosses were made but failed to produce viable seed. The poor results of the present season's crossing apparently were due to scanty plant growth and continual dieback of the growing stem tips. This did not appear to be caused by insects or disease and may possibly have resulted from repeated application of DDT sprays to control sweetpotato weevils and other insect pests. Fifteen new sweetpotato introductions, including two backcrosses, eleven 3-way crosses, and two varieties, were received from the Bureau of Plant Industry, Soils, and Agricultural Engineering. All but the last are selections from crosses made at this station during the 1950–51 season.

SWEET CORN BREEDING. L. A. Snyder, E. Cabanillas, and H. J. Cruzado.

Sixth-generation inbreds of USDA–34 sweet corn exhibited good uniformity and vigor. Five S_6 lines of USDA–34 have been grown and advanced to the seventh generation. After five generations of vigorous selection for insect resistance, yield, vigor, and normal flowering, these lines show a low incidence of segregation and unfavorable genes. All of the S_6 lines showed satisfactory resistance to corn

earworm (*Heliothus armigera* Hbn.). An additional 48 S_5 lines were grown, 32 of which have been advanced to the sixth inbred generation; and 35 S_4 lines were grown, 26 of which have been advanced to the fifth generation. Major factors accounting for the discarding of lines were susceptibility to corn earworm, dwarfing, abnormal flowering (failure of anthesis or anthesis delayed until the silks were no longer receptive), or breaking of the stalk above the upper ear. Appreciable segregation was observed in some of the fifth generation lines and in most of the fourth generation lines.

MACADAMIA SEED. W. C. Kennard and F. Llavat-Cristy.

Seed of *Macadamia ternifolia* F. Muell. were received from Dr. J. H. Beaumont, horticulturist of the University of Hawaii. Five pounds of seed were sent to the Virgin Islands Research and Extension Program for germination and test there. The remainder of the seed was divided on the basis of appearance as follows: Best, good, poor, and flat sided. Some of the best seeds were scarified on an emery wheel to facilitate the penetration of water through the thick and impervious seed coat. Periodic counts on emergence showed that the best germination occurred within 2 to 4 months after planting, but a few seeds germinated as late as 5 months after planting.

The data showed that only the best appearing seed should be selected for germination and that scarification as was done in this case was detrimental to germination.

PLANT INTRODUCTION AND PROPAGATION

PLANT INTRODUCTION. H. F. Winters, N. Almeyda, and F. Rodríguez-Colón.

Three hundred and sixteen introductions were received from 14 foreign countries and from the United States and Territories. Of particular interest were several introductions of tropical fruit including two varieties of the rambutan, *Nephelium lappaceum* L.; the Brewster variety of *Litchi chinensis* Sonn. which has given good yields in Florida; and Hume's variety of *Lucuma nervosa* A. DC., the canistel or eggfruit. Eighteen species of *Strophanthus* were added to the collection received during the past few years. Forty-five plants of *Anthurium* spp. were sent by a correspondent who had collected them from the wild in Colombia. Several varieties of sweetpotatoes and a considerable number of forage plants, both legumes and grasses, were received. Seed was received from El Salvador of *Simarouba glauca* DC. which yields an edible semisolid fat. Four introductions of black pepper, *Piper nigrum* L., were received from the Bureau of Plant Industry, Soils, and Agricultural Engineering, Glenn Dale, Md.

DISTRIBUTIONS. H. F. Winters, N. Almeyda, and F. Rodríguez-Colón.

A total of 352 packets of seed were sent to 14 foreign countries and to the United States and possessions during the year. Considerable interest has been shown in obtaining seed of the coffee varieties maintained in the station collection.

Local demand for plants, particularly ornamentals, continued great. A total of 9,978 ornamental plants and trees, 637 fruit trees, and 80 square feet of Zoysia sod were distributed locally. Plants of black pepper, *Piper nigrum* L., totaling 669, were sent to Costa Rica, Ecua-

dor, Guatemala, and Nicaragua. These plants were propagated asexually from material obtained in Bangka, Indonesia.

The durian, *Durio zibethinus* Murr. fruited for the first time in several years. One of the two mature trees gave 15 medium-sized fruit. An inflorescence has developed on the talipot palm, *Corypha umbraculifera* L., a giant fan-leaved palm from Ceylon which flowers only at maturity. The palm is reported to die after ripening the seed which may require 2 years.

HEVEA FRUIT SIZE. H. E. Warmke.

Measurements were made on ovaries of a large number of open-, self-, and cross-pollinated flowers of *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg., at given intervals after anthesis. These indicated that ovary size at anthesis (average 2.1 mm. in diameter and 2.7 mm. in length) was maintained within narrow limits until the 14th to 24th day. Between the 14th and 18th day, approximately 90 percent of the blossoms dropped from the trees, without undergoing appreciable change in size. The remaining 10 percent began to enlarge on the 24th day and either dropped (approximately one-half) or continued to enlarge at a rapid rate until the 90th day, when mature fruit size was reached. Thus 90 percent of the ovaries dropped without any enlargement and only approximately 5 percent grew through to maturity. It is of further interest that length is greater than diameter in young fruits. The rate of increase differs, however, so that diameter and length are equal at approximately 30 days, and in the older fruits diameter usually exceeds length.

HEVEA FRUIT SET. T. J. Muzik and H. E. Warmke.

The scientific breeding of *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg., the Para rubber tree, has been handicapped by the loss of 90 to 95 percent of the fruits between pollination and maturity. Numerous growth-regulating chemicals were sprayed on the flowers and young fruits to determine if they would affect fruit set or retention. The chemicals used included parachlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, chlorophenoxypropionic acid, 2,4-dichlorophenoxypropionic acid, 2,4-trichlorophenoxypropionic acid, butyl 2,4,5,-trichlorophenoxyacetate, napthalenacetic acid, and 2,3,5-triiodobenzoic acid. A solution of Nu-Green (urea) and sugar was also tested.

No consistent improvement in fruit set was obtained from any treatment of the flowers. In the treatment of the young fruits, triiodobenzoic acid gave results somewhat superior to the control. Fruits in this treatment were smaller and lighter in color and the seeds from all of the sprayed fruits and particularly those sprayed with triiodobenzoic acid were lighter in weight, but germination and early growth were excellent in all treatments.

MONOCOT GRAFTING. T. J. Muzik.

Some of the most conspicuous plants in the tropical landscape are the large monocotyledonous lianas. Using a modification of the method previously described on the large grasses, some of these lianas including *Scindapsis aureus* (Lind.) Engler, *Nephthytis afzelii* Schott, and *Philodendron dubium* Chod. & Vischer, were grafted. The young stems were broken in the meristematic region. A waxed paper

tube was slipped over the stock, and the scion inserted and tied firmly. Union of stock and scion was followed by the same general pattern as in the other monocots, but cell division appeared to take place at a greater rate and for a longer time. In the initial stage, there was a tenfold enlargement of the cells adjacent to the contact layer on both scion and stock. These cells were then divided to form approximately 24 to 30 tiers of new cells as compared with 3 to 5 tiers in the grasses. Differentiation of vascular tissue was not completed for several months. After that time, however, growth was rapid and some scions have attained a length of several feet. It is significant that the grafting of monocots has been extended to families other than the Gramineae and that the pattern of union is the same in many respects. These observations suggest that many other types of monocotyledonous plants may also be grafted and that vascular union will follow the same general sequence, i. e., formation of a contact layer, cell division along the edges of stock and scion, and then differentiation of these new cells into tracheids to reunite the original vascular bundle.

BAMBOO

Bamboo Distribution. W. C. Kennard and F. Llavat-Cristy.

During the year 70 branch cuttings and 7 rhizome cuttings of *Dendrocalamus asper* (Schultes) Backer, and 4 rhizome cuttings of *Bambusa multiplex* (Lour.) Raeusch. var. Alphonse Karr were distributed in addition to 189 plants of *Bambusa tulda* Roxb., 111 of *Gigantochloa apus* (Roem. & Schult.) Kurz ex Munro, 68 of *Bambusa tuldoides* Munro, 20 of *Bambusa ventricosa* McClure, 15 of *Arundinaria amabilis* McClure, 15 of *Bambusa textilis* McClure, 8 of *Bambusa longispiculata* Gamble ex Brandis, and 5 of *Dendrocalamus strictus* Nees.

In cooperation with the Puerto Rico Industrial Development Co., 6,141 linear feet of cured *Bambusa tulda* culms, 30 linear feet of cured *Bambusa textilis* culms, and 250 pounds of *Bambusa tulda* side branches were made available to continental and local bamboo industries.

Bamboo Harvesting. W. C. Kennard and F. Llavat-Cristy.

In January 1949 an experiment was initiated to compare the effect of eight harvesting treatments on yield and quality of *Bambusa tulda* planted 25 feet apart on the square on Catalina clay soil. The variables in this experiment are (1) age of plants when harvesting is begun, (2) harvesting cycle, and (3) age of culms when cut. The objectives of the experiment are to determine the effect of the different treatments on (1) the development of the plant, (2) the quality and yield of culm material, and (3) the labor (cost) of harvesting. This experiment also produces culms documented as to age for use in other studies. Through the 1952 harvest the 3-year-old culms had the largest diameter and the greatest length. In 1953, however, the 4-year-old culms exceeded slightly the length and diameter of the 3-year-old culms, indicating that the clumps may have reached the age when new culms are approximately the same size each year. Since this experiment is designed to run for several more years, no valid conclusions can be drawn from the data obtained to date.

BAMBOO PROPAGATION. F. A. McClure,[1] F. Llavat-Cristy, and W. C. Kennard.

Experiments conducted to find a means of producing large numbers of rooted plants that could be removed easily and transported to planting sites yielded the following results.

Propagation by means of cuttings consisting of "easy-to-get" rhizomes found near the soil surface around clumps of many of the species, has proved to be a reliable, though not prolific, source of small rooted plants. The recommended time for obtaining these propagules is just at the end of the dry season, before their buds have begun to push. Dipping the freshly dug rhizomes for 5 minutes in a 3-percent solution of commercial chlorox, followed by sealing the cut ends with melted paraffin, was effective in reducing the losses in rhizomes of *Bambusa tuldoides* and *B. textilis*.

Propagating *Dendrocalamus strictus* and *Bambusa tulda* by the method of clump layers gave negative results. From two clumps of *Bambusa longispiculata*, 150 small, rooted culms were obtained in 9 months. It is believed that this method, or some modification of it, may be developed for propagating some species.

The propagation of *Arundinaria amabilis*, the species known as Tonkin cane used for split-bamboo fishing poles, has proved to be very difficult. Under conditions of culture available at present, the most dependable method of increasing this species is by heavy fertilization of the mother plant, and dividing the rhizome system once a year at the end of the dry season. The young plants grew much better in heavy clay than in cocopeat and they also responded well to an application of two ounces of complete fertilizer per plant as soon as they were well established.

Plantings of this species put in 2 years ago at Toro Negro (elevation 3,500 feet) and at Maricao (elevation 2,600 feet) seem to be establishing themselves more rapidly than those planted at the same time at Mayaguez (50 feet).

The most satisfactory means of propagating several of the bamboo species to date is by whole culm cuttings. Preliminary results indicate that the different species respond differentially to various propagating media. The following observations were made concerning propagation by this method: (1) The oldest material gives the best results so long as viable buds are present, (2) culm cuttings should be put in as close as possible to the beginning of the rainy season, (3) special care should be taken to prevent drying of the culms or their buds between the time they are cut and the time they are covered with earth, and (4) it takes at least one full year for the satisfactory development of the yield of a culm.

VANILLA

VANILLA CULTURE. T. Theis and F. A. Jiménez.

The mulch beds used in the lath shade method of growing vanilla, while providing good growth in the absence of root rot causes several conditions adverse to the plant when the disease is present. Vanilla plants grown under lath shade are supported by one or several bucare

[1] Technical Collaboration Branch, Office of Foreign Agricultural Relations, U. S. Department of Agriculture.

trees. The root system of these plants consists of aerial roots which extend from the vine to the mulch. These roots may grow freely from the plants to the ground but are more frequently attached to the trunk of the support trees. When these roots enter the mulch, they branch and spread throughout the surrounding area. Thus, a condition exists in which the plants are separated from the growing medium by a distance of several feet. The vines are connected to the root system by a relatively small number of main roots concentrated about the trunk of the support tree, a situation favorable for root rot development.

A new method of vanilla culture was initiated in an effort to stimulate root dispersal and to produce conditions less favorable for the disease. A bed was established in a lath shade house with four bucare posts planted in the form of a rectangle (1½ x 6 feet). Galvanized wire fencing was stretched inside the posts. This, in effect, made a wire box 1½ x 6 feet. The box was then filled with grass mulch and eight node vanilla cuttings were planted about the base. The basal ends of the cuttings were placed under the mulch. In the past 20 months growth has been excellent in the new type bed. The leaves are dark green and turgid and stem girth is thick. The aerial roots were in contact with the mulch soon after they emerged. Some of them grew along the outside of bed and ultimately reached the soil and developed root hairs wherever they contacted the mulch. Other roots have penetrated the mulch. This technique provides a means for stimulating the dispersal of roots thus creating a condition less favorable for the spread of root rot.

VANILLA HYBRIDS. T. Theis and F. A. Jiménez.

The most practical method for the control of vanilla root rot is by the selection or breeding of a disease-resistant plant with good fruit quality. In 1943 crosses between the susceptible commercial species of vanilla, *Vanilla planifolia* Andr. (*V. fragrans* (Salisb.) Ames) and the resistant noncommercial *V. phaeantha* Reich. were made. During the 1951 flowering season, three of the hybrids blossomed and bore fruit for the first time. The hybrid pods were cured and tested for quality. Only the lead number was within expected limits. The vanilla content was low and the figure for total solids was high. There was a heliotropin odor in the extract similar to that obtained from Tahiti vanilla.

The comparison of the hybrid plants with the parental types indicated a true crossing. The leaf types found in the hybrids include the parental types and all gradations. The flower color, size of floral parts, and pod size are intermediate. Some plants have the type of root growth and apparent root resistance of *Vanilla phaeantha*. Others resemble *V. planifolia* in their susceptibility to the disease.

Although it appears that the root rot resistance of *Vanilla phaeantha* has been transmitted to some of the hybrids, these plants have not produced a heavy crop of fruit. Since heavy production and root rot resistance appear to be correlated, the resistance of the hybrids is still not proved.

VANILLA BACKCROSSES. T. Theis and F. A. Jiménez.

Since the preliminary tests indicated that the hybrid pods were not of sufficiently good quality for commercial use, backcrosses with *Vanilla planifolia* are therefore necessary to improve quality. Back-

crosses of the hybrid plants to *V. planifolia* were made when the hybrids first flowered. The seeds from this cross were harvested and germinated using the recommended aseptic techniques. When the backcrosses were 17 months old, they were potted and transferred to a small greenhouse, where they have remained green and healthy. Within a year or so these will be transplanted to the field.

CHEMOTHERAPEUTANTS FOR ROOT ROT CONTROL. T. Theis and F. A. Jiménez.

One of the newer approaches to plant disease control is the use of chemotherapeutants, chemicals that are applied to the soil and absorbed by the plant. Since no disease inhibition was observed at the concentrations of two chemotherapeutants previously used, further studies were carried out to find concentrations that might be effective.

Two-node rooted cuttings of *Vanilla planifolia*, some of which were deliberately injured by removing a thin slice of the root near the tip were inoculated by dipping the roots in a spore-mycelium suspension of the root rot fungus and then planted in wooden boxes.

The two compounds tested were 4 chloro 3,5-dimethylphenoxyethanol (Code No. 1182) and 2-norcamphane methanol (Code No. 1207). The former compound was applied at the rate of 0, 15, 32, 63, 125, and 250 p. p. m. The latter was applied at the rate of 0, 63, 125, 250, 500, and 1,000 p. p. m. Each solution was added to the plants at the rate of 250 cc. per box, twice a week. The chemotherapeutants were applied two weeks previous to the date of inoculation and continued until the experiment was terminated.

There was a significant reduction in the incidence of the disease obtained with compound 1207 at 63 p. p. m. and higher.

Compound No. 1182 caused root injury at a concentration of 250 p. p. m. and no disease reduction was observed at any of the concentrations tested.

VANILLA MULCH. T. Theis and F. A. Jiménez.

One of the contributing factors favoring root rot has been attributed to a possible lowering of the pH by the liberation of organic acids as the mulch decomposed. This theory has been accepted almost as fact without experimental proof.

Over an extended period of time pH measurements were made of the mulch in an experiment to test the effect of mulch fumigants and sanitary practices for vanilla root rot control. The mulch was composed of a mixture of native grasses which included a small number of native legumes and other broadleaved plants. The samples for pH determination were selected in an area of root growth, below the surface, about 1 foot in radius around the base of the plant. Although there were variations in the pH of the mulch from the different treatments, these differences were not great enough to support the concept that an old mulch becomes acid and favors root rot.

COCONUTS AND MANGOES

DWARF VS. COMMON COCONUTS. N. Almeyda and H. F. Winters.

An experiment was started 2 years ago to compare the yielding ability of a dwarf coconut variety and the variety commonly grown in Puerto Rico. The experiment consists of 5 replications of the 2

varieties, arranged in alternate plots, each consisting of 12 trees spaced 25 x 25 feet.

Records to date of the diameter of the trunk at 3 inches from the ground level, the length of the trunk, and the length of the largest leaf showed that there was no statistical difference in growth between the two types of coconuts. However, the trend of the data suggests that some differences may be expected in subsequent years. In general, all the plants appear healthy. The dwarf coconut leaves are orange in color, whereas the common coconut leaves are deep green. The difference in color is probably not due to a mineral deficiency, but most likely to a difference in genetic constitution.

MANGO PROPAGATION. N. Almeyda.

Propagation of the mango by the old inarching practices used by nurserymen in India takes from 2 to 3 years before the grafted plant is ready for transplanting to the field. Singh [2] has shown that mango seeds planted in the first week of July, and inarched in early September, could be detached from the mother tree by the end of September. Only 3 months elapsed from the time the mango seeds were planted until the grafts were detached.

An attempt was made to reduce the time needed for commercial propagation of mango by inarching. One-month-old seedlings were tied to different mango varieties by string and inarched. One month later the grafts were detached from the parent tree and potted.

Although dry weather followed the inarching operation, 78 percent of probable success was obtained. Greater success might be obtained if the operations were carried out during a rainy period.

MANGOSTEEN TRANSPLANTS. H. F. Winters and F. Rodríguez-Colón.

The survival of mangosteen seedlings when transplanted to the field has been notably low. An experiment was conducted to determine if watering mangosteen transplants during the dry season or spraying the foliage with a latex material prior to transplanting would improve the survival.

Two-year-old mangosteen seedlings were transplanted to a prepared field located in a humid valley with a high water table. Half of the plants were watered as often as necessary to keep soil moisture high during the dry season following transplanting. The other half received only the water from rain. One half of the trees in each of the main plots were sprayed the day before they were dug with a 50 percent solution of Goodrite latex, a synthetic preparation which reduces water loss by transpiration. The other half of the trees in each plot were not sprayed. After 1 year, the plants receiving the foliage spray showed 92 percent survival and those not sprayed showed only 73 percent survival. The trees receiving water during the dry season showed an average survival of 71 percent, whereas those not receiving supplementary water showed a survival of 94 percent, an indication that the additional water was detrimental under the conditions of the experiment.

From this experiment it appears that under humid soil conditions with high water table additional irrigation, even during the dry season, is detrimental to survival of mangosteens but that spraying with Goodrite latex is beneficial.

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[2] SINGH, L. F. MANGO GRAFTING IN 8 WEEKS. Science 114: 393, illus. 1951.

WEED CONTROL INVESTIGATIONS

EFFECT OF 2,4-D ON PHOSPHOROUS METABOLISM. A. J. Loustalot, M. P. Morris, J. García-Rivera, and C. Pagán.

The mode of action of 2,4-D on susceptible plants is not well understood. A preliminary experiment in which two species of plants, *Commelina* sp. and *Xanthosoma* sp., were analyzed 24 hours and 1 week after being treated with 2,4-D showed that the percentage of water-soluble phosphorus in treated plants was consistently higher than in untreated plants. In view of these results a large-scale replicated experiment with a uniform variety of plants was carried out to obtain additional information on the effect of 2,4-D on phosphorous metabolism. A variety of white beans known as Blanca Bonita (P. R. No. 1632) was planted in a field divided into 12 plots, each 52 x 24 feet. An experimental design was imposed on the field consisting of 4 randomized blocks with 3 plots each.

When the plants were 5 weeks old one plot in each replication was sprayed simultaneously with 0.1 percent sodium 2,4-D. The plants in another plot of each replication were uprooted at the same time and were left lying on the ground. The third plot in each replication was left as a control. Samples of 100 plants were taken from all plots at 4, 10, 24, and 48 hours and 1 week after treatment and analyzed for inorganic and organic phosphorus.

In general the fluctuations of organic P in the roots of treated plants was opposite to that of the inorganic fraction, i. e., when organic phosphorus was low in the treated roots inorganic P was high and vice versa. This indicates that the changes in metabolic processes of the plant induced by 2,4-D probably caused the shift in phosphorus from one form to another. There was no such complementary effect or fluctuation between organic and inorganic phosphorous fraction in the roots of check or uprooted plants. The level of inorganic P in leaves and stems of treated plants fluctuated in most instances like that in the check plants, but this fraction was consistently higher at all sampling dates in roots, stems, and leaves of treated plants.

The data obtained are considered as positive if not conclusive evidence that 2,4-D induces an irreversible hydrolysis of one or more of the high energy phosphates. Presumably, once the inorganic phosphorus is split off from the high energy phosphate molecules the enzyme or enzyme system needed for transferring it again to the organic molecule is "poisoned" and therefore it does not reenter into the metabolic cycle as it normally would. Since the release of energy from the high energy phosphate compounds is essential for the synthesis and utilization of carbohydrate (hexose phosphates), an interruption of the transfer of energy through the phosphate bond would produce drastic and lethal effects in the plant metabolism. Loustalot and Muzik [3] have shown that photosynthesis is stopped or reduced when very small amounts of 2,4-D are applied to velvetbeans. Other workers have shown that the rate of respiration is increased in plants treated with 2,4-D. It has also been reported that starch is depleted and the sugar content increased as a result of 2,4-D applica-

[3] LOUSTALOT, A. J., and MUZIK, T. J. THE EFFECT OF 2,4-D ON APPARENT PHOTOSYNTHESIS AND DEVELOPMENTAL MORPHOLOGY OF VELVET BEANS. Bot. Gaz. 115. 1942. [In press.]

tion. These important physiological reactions are all controlled in one way or another by energy transfer from the hydrolysis of high energy phosphate linkages. The data obtained in the present experiment suggest that the mechanism of 2,4-D action is probably through the inhibition or interruption of the phosphate metabolism in the plant.

EFFECT OF 2,4-D ON NUTGRASS. T. J. Muzik and H. J. Cruzado.

No clonal resistance to 2,4-D was found in nutgrass (*Cyperus rotundus* L.). The ability of plants to recover from treatment with 2,4-D is due to the fact that only the sprouts are killed while the tubers below ground remain viable. Alternate plowing and spraying with 2,4-D was effective in reducing the nutgrass population about 90 percent, although after 16 months sufficient tubers remained to reinfest the area. Plowing alone increased the infestation about 68 percent.

EFFECT OF CMU ON NUTGRASS. A. J. Loustalot and H. J. Cruzado.

Field experiments to control nutgrass with CMU (3-parachlorophenyl-1,1 dimethylurea) have given good but sometimes inconclusive results. Since soil moisture levels, depth at which tubers are located in the soil, number and physiological condition of tubers, and many other factors vary widely in relatively small areas, it is not surprising that results of field experiments are sometimes difficult to interpret. An experiment designed to study the effect of CMU on nutgrass tubers at different depths in the soil was carried out under greenhouse conditions where environmental factors could be better controlled.

Tubers located 1 to 8 inches below the surface of untreated soil increased 9- to 11-fold during 6 months. Those planted 10 to 14 inches below the surface of untreated soil increased 7- to 8-fold during the same period. All tubers appeared healthy regardless of depth of planting, but the new tubers developed from those planted 1 to 8 inches below the surface were larger and heavier than those developed from tubers planted 10 to 14 inches deep.

The tubers planted 1 and 2 inches below the surface of soil treated with CMU at 40 pounds per acre were completely eradicated and those planted at a 4-inch depth were very small and emaciated. Tubers planted 6 to 12 inches below treated soil showed considerable decrease but some were still viable after 6 months. As the planting depth increased the size and weight of the surviving tubers increased. Those located 14 inches below treated soil increased 3-fold in number, and were healthy though somewhat smaller than the corresponding check tubers.

An experiment conducted last year showed that high rates (80 pounds per acre) of CMU greatly reduced the population of nutgrass tubers in the soil but did not eradicate them. Subsequent field and greenhouse studies on the movement and persistence of CMU in the soil indicated that the reason for the incomplete eradication was due to the fact that CMU remained near the surface of the soil regardless of the amount of rainfall. Thus, the herbicide did not come in contact with the tubers located deep in the soil and they remained viable. In view of this fact an experiment was planned in which relatively low rates of CMU was combined with alternate plowings to bring the nutgrass tubers in contact with the compound. Plots treated with 2,4-D and TCA were also included in the experiment for comparison.

Six months after the initial treatment all plots in which CMU was

applied either alone or in combination with 2,4-D and TCA had practically no live tubers remaining. On the other hand, check plots and those treated with TCA at 40 pounds per acre had more than 600 tubers per cubic foot and plots treated with 5 pounds per acre of 2,4-D had 250.

PERSISTENCE OF CMU IN SOIL. A. J. Loustalot and H. J. Cruzado.

Experiments were conducted to study the effect of temperature, soil moisture, and soil texture on the persistence of CMU in the soil.

Flats of soil treated with 0, 1, 5, and 10 pounds per acre of CMU were stored for 0, 2, 4, and 10 weeks under various experimental conditions before being planted to corn and velvetbeans.

The toxicity of CMU, as measured by the growth of corn and velvetbeans in treated soil, persisted longer at 10° C. than it did at room temperature or at 45° C. There was no appreciable difference in persistence of the herbicide between the two warmer temperatures. As would be expected, the toxicity was more prolonged at the higher rates of application at all temperatures. Soil treated with CMU at the rate of 5 pounds per acre and stored at 10° C., remained toxic to corn and velvetbeans for the duration of the experiment (10 weeks). Soil treated at the same rate but stored at room temperature or 45° C., lost its toxicity to the beans within 2 weeks and to corn within 10 weeks.

At the 1-pound-per-acre rate, the toxicity of CMU was dissipated within 2 weeks after application at all moisture levels. At the 5-pound rate 2 to 4 weeks were required for the toxicity to disappear in soil with a medium or saturated moisture level, but at least 10 weeks were required for the toxicity to disappear in dry soil. At the 10-pound rate, the CMU toxicity had dissipated within 10 weeks in medium and saturated soil but was still present in air-dry soil at the end of that time.

Sandy soil retained the toxicity of CMU longer than did the heavier soils with a higher clay content. The toxicity at all levels of application had practically disappeared in clay soils within 10 weeks, whereas in the sandy soil the toxicity still persisted at the end of that time.

PERSISTENCE OF CMU UNDER FIELD CONDITIONS. A. J. Loustalot and H. J. Cruzado.

An experiment to determine the persistence of CMU under field conditions showed that corn planted immediately after treatment in plots sprayed with 1 pound per acre was not injured but velvetbeans in these plots were affected until the third planting which was made 2 weeks after treatment. Plots treated at the 5-pound-rate remained toxic to both corn and velvetbeans for 4 months. Plots treated at the 10-pound-rate remained toxic to corn for 6 months and to velvetbeans for 8 months. Soil treated with 20 pounds per acre of CMU remained toxic for 8 months to both test crops. After 12 months the soil treated with 80 pounds per acre of CMU was no longer toxic to corn but it was toxic to velvetbeans as evidenced by injury symptoms and stunting. The data obtained in this experiment show that CMU persists in the soil for long periods of time, even when applied at relatively low rates, and for that reason it should be used with extreme caution. The data also indicate that corn and presumably other members of the grass family are more resistant to CMU than velvetbean and other legumes or broadleaves.

MOVEMENT OF CMU IN THE PLANT. T. J. Muzik and H. J. Cruzado.

Absorption and translocation studies showed that CMU entered plants through leaves, stems, or roots. It entered more rapidly through the ventral leaf surface than through the dorsal surface. Plants could be killed either by dipping the tops in CMU solution or by applying CMU to the roots only. Translocation from leaves to stems occurred very slowly. Movement of CMU in the plant is primarly upward and takes places mainly through the xylem. Entry and translocation were very rapid in roots. When one-half of the root systems of velvetbean plants (*Stizolobium deeringianum* Bort.) were placed in CMU solution (200 p. p. m.) for 15 minutes or more, the plants were killed. Root tips were grown for 3 months in sterile nutrient containing CMU at 10 and 25 p. p. m., although intact plants were killed when their root systems were placed in similar autoclaved solutions, indicating that CMU acted primarily on the leaves and stems.

EFFECT OF CMU ON MORPHOGENESIS. T. J. Muzik and H. J. Cruzado.

There is little or no published information on how CMU exerts its action on plants, although it has become widely used as a nonselective herbicide. Some herbicides, such as 2,4-D, cause extensive tissue modifications. The effect of CMU on the morphogenesis of velvetbeans (*Stizolobium deeringianum*) was measured by adding the CMU (50 cc. of 200 p. p. m. solution) to the soil in each pot in which the plants were growing. Yellow spots, which soon turned black, appeared between the large veins on the old leaves 10 to 14 days after treatment. Later these necrotic areas spread to the margins of the leaf. Although the older leaves were affected first, the plants died progressively downward from the top with the stems and leaves turning black and wilting.

Sections through the affected regions showed that many of the cells of the epidermis, cortex, and phloem parenchyma had collapsed. This suggests that the wilting of the stem is due to a loss of turgor pressure in these cells. Since no abnormal cell proliferation or tissue modifications were observed, the action of the CMU is apparently very different from 2,4-D which caused extensive abnormal cell proliferation in susceptible plants.

CMU VOLATILITY. T. J. Muzik and H. J. Cruzado.

It has been well established that sensitive plants may be severely affected by the volatilization of some chemicals, such as the butyl and methyl esters of 2,4-dichlorophenoxyacetic acid. From a herbicidal standpoint knowledge of the volatile characteristics is important in determining the likelihood of injury to plants near the treated area.

Young pigeonpeas (*Cajanus cajan* (L.) Druce) were grown in gastight bell jars. Four plants were placed in each chamber containing a beaker with one of the following: 1 gram of CMU powder, 50 cc. of 0.1 percent suspension, 50 cc. of a 200 p. p. m. solution of 50 cc. of water. No toxic effect was noted until 25 days had elapsed, indicating that the CMU was entering the atmosphere very slowly if at all. At that time the leaf immediately above the beaker containing the CMU powder became yellow and spotted. Fifteen days later this plant died. However, by this time even the foliage on the control plants had turned somewhat yellow, probably due to poor circulation of air in the chambers.

Compared to the high volatility of many 2,4-D esters which cause epinasty within an hour, CMU in the form used volatilizes very slowly.

HERBICIDE EVALUATION. H. J. Cruzado, A. J. Loustalot, and T. J. Muzik.

Four new chemicals, N–1 naphthyl phthalmic acid at 2, 5, and 10 pounds per acre, sodium isopropyl xanthate (Nix) and Oktone (40 percent saturated solution of octachlorocyclohexenone in petroleum solvent) each at 8 pounds per acre, and XTB (composition not disclosed) at 7 pounds per acre were tested as preemergence and postemergence herbicides. Corn as a representative of the grass family and velvetbeans as a representative of the legume family were used as test crops.

N–1 naphthyl phthalmic acid at 10 pounds per acre was effective in controlling most of the important weed species present, except nutgrass. However, at that rate the germination and growth of corn was severely reduced. Even at the 5-pound-per-acre rate the germination and growth of corn was adversely affected and weed control was poor. Germination of velvetbeans was normal at all rates of N–1 naphthyl phthalmic acid, but subsequent growth was stunted even at the 2-pound-per-acre rate. The compounds Nix and XTB both gave good control of portulaca species but only fair to poor control of the other weed species, with the exception of amaranthus, which was controlled by XTB. The germination of corn and beans was not appreciably affected by either Nix or XTB at the rates used. However, subsequent growth of both the test crops showed abnormalities in the plots treated with XTB. Growth of the crops in the Nix plots seemed normal, although yield of fresh matter in both XTB and Nix plots was somewhat reduced.

Oktone gave the best results of the four new herbicides tested. With the exception of nutgrass, the control of weeds was good to excellent in these plots. In addition, germination and growth of the test crops was normal, and the yield of fresh material was the highest of all the plots.

The herbicidal properties of the four chemicals were tested on established stands of mixed weed infestations. The toxicity of the compounds to growing corn and velvetbeans was also tested by spraying them on established stands of these crops.

N–1 naphthyl phthalmic acid at the rate of 10 pounds per acre caused burning of the corn tops but had no apparent effect on the velvetbeans. Established stands of *Amaranthus*, *Ipomoea*, *Bidens pilosa*, *Portulaca*, and kenaf species were effectively controlled, but members of the grass family and nutgrass were not affected, indicating that this material has potentialities as a selective herbicide. The compound designated Nix (sodium isopropyl xanthate) caused slight burning on corn and beans, but it effectively controlled poinsettia, Amaranthus, and Ipomoea species. It also had no effect on the grasses. The compound XTB caused severe burning of both corn and beans and controlled only one of the weed species present, *Bidens pilosa*. Oktone effectively controlled *Poinsettia*, *Amaranthus*, and *Ipomoea* species and killed nutgrass shoots. It had no appreciable effect on the grasses.

Nalco herbicides RJL 19 and WES 101 appeared to be selective for corn although they caused severe damage to pigeonpeas and velvetbeans when applied as preemergence treatments. The pigeonpeas and

velvetbeans were stunted and the leaves were curled but the corn was normal in growth and appearance.

BREEDING AND EVALUATION OF FORAGE CROPS

GRASS SEGREGATION. H. E. Warmke.

More than 3,500 offspring of three varieties of guineagrass (*Panicum maximum* Jacq.) were grown in progeny tests during the year. The purpose of the tests was to approximate, by observing the number of exceptional plants, what portion of the offspring was sexual in this species which previously had been shown to be highly apomictic. Two forms of common guineagrass (one from Mayaguez and one from Isabela) produced 4.7 and 2.6 percent exceptional plants, respectively. Eleven hundred seedlings of the variety gramalote produced only 1.3 percent exceptional plants. These variant plants differed in size, vigor, leaf shape, color, time of maturity, etc., from the otherwise uniform populations. These tests indicate that the amount of true sexual reproduction in these varieties of *Panicum maximum* is small.

GRASS CYTOLOGY. L. A. Snyder.

Chromosome numbers were determined in 14 previously unrecorded grass species. The species studied, the somatic chromosome numbers and maximum chromosome association at first meiotic metaphase (in parenthesis) are as follows: *Andropogon brevifolius* Swartz, $2n=40$ (20_{II}), *A. intermedius* R. Br., $2n=60$, *Hyparrhenia hirta* (L.) Stapf, $2n=40$ ($2_{IV}+16_{II}$), *H. rufa* (Nees) Stapf, $2n=40$ ($1_{IV}+18_{II}$), *Sorghastrum setosum* (Griseb.) Hitchc., $2n=20$ (10_{II}), *Paspalum virgatum* L., $2n=40$ (20_{II}), *P. secans* Hitchc. & Chase, $2n=40$ ($3_{II}+34_{I}$), *P. malacophyllum* Trin. $2n=40$ ($4_{IV}+12_{II}$). *P. millegrana* Schrad. $2n=41$ ($19_{II}+1_{III}$), *P. macrophylum* H. B. K., $2n=60$ (30_{II}), *P. laxum* Lam., $2n=60$ (30_{II}), *P. lividum* Trin., $2n=70$ ($9_{IV}+3_{III}+14_{II}$), *Pennisetum ciliare* (L.) Link. $2n=36$ ($4_{IV}+10_{II}$), *Setaria geniculata* (Lam.) Beauv., $2n=36$ (18_{II}).

TROPICAL KUDZU BREEDING. H. E. Warmke.

For several years this station has been interested in attempting to cross *Pueraria phaseoloides* (Roxb.) Benth. (tropical kudzu) with *P. thunbergiana* (Sieb. & Zucc.) Benth. (States kudzu). This has been difficult because the former flowers during the winter months, while the latter flowers during the summer. This past year it was learned that both species flower simultaneously (June, July, and August) in the Southern United States and in South America. Furthermore, 24- to 48-hour air service is now available between the two areas. It seemed worth while, therefore, to try transportation of pollen in an attempt to obtain crosses. The cooperation of two workers in South America (from Brazil and Peru) and three in the Southern United States was solicited. Several shipments of flowers and of pollen were made to both South American stations, but no pollen arrived in condition good enough to make successful crosses. Local postal systems and plant inspections delayed the shipments from 6 to 30 days.

LEGUME EVALUATION. R. H. Freyre and H. E. Warmke.

A group of 26 legume species from Central America was received from the Division of Plant Introduction and Exploration, BPISAE.

These included *Centrosema*, *Cicer*, *Dolichos*, *Galactia*, *Indigofera*, *Phaseolus*, and *Vigna* species. All were grown in test plots in the south field, and their behavior, yield, and growth habits were recorded.

LEGUME ESTABLISHMENT. H. E. Warmke and R. H. Freyre.

Tests were made during the year to determine if tropical kudzu and trailing indigo could be started in established stands of Merkergrass (*Pennisetum purpureum* Schum. var. *merkerii* Hort.). A recent experiment at this station showed a significant advantage in forage production and in protein and mineral yields of combinations of these legumes with Merkergrass, as compared with either grass or legumes grown alone. In this experiment, however, the grass and legumes were planted at the same time in clean-tilled plots. Since there are many acres of Merkergrass already in production on the island, it was of interest to know if these could be converted to grass-legume associations by establishing legumes among the old grass plants.

Rooted cuttings of trailing indigo and established seedlings of tropical kudzu were planted at 2-foot intervals between the rows of 3-year-old Merkergrass (cut just prior to transplanting of legumes). Two months later, the grass was again harvested, to prevent excessive shading of the young legume plants. At the end of 1 year the grass and legumes were harvested from the newly established plots and the yields compared with those of comparable 3-year-old grass-legume associations. The new plots produced an average of 2,605 pounds/acre of green kudzu forage as compared with 2,125 pounds/acre for the old. Trailing indigo in the new plots yielded an average of 1,800 pounds/acre; in the old plots it produced only 1,080 pounds/acre. These tests indicate that, with good management, trailing indigo and tropical kudzu can be established successfully in old stands of Merkergrass.

LEGUME CROSSING TECHNIQUES. L. A. Snyder and E. Cabanillas.

Hot water emasculation of *Indigofera* flowers has been attempted at the station during the year. This technique was originally devised to facilitate hybridization of small-flowered grasses. It involves immersing inflorescences of intended female parents in hot water for several minutes, a day or more before anthesis would occur, taking advantage of the fact that the female parts of the flower withstand a higher temperature than does the pollen. The major advantage of the method lies in the fact that the flowers are not mechanically injured or disturbed. Materials used in the present trials were a pint thermos bottle for treatment, a quart thermos bottle to hold a supply of water, a thermometer, and an interval timer. Temperature changes in the pint thermos bottle during treatment depend upon wind and air temperature, but never exceed one-half of a degree (centigrade) during a 5-minute treatment. The flowers used were those which opened the morning following hot-water treatment.

Immersion for 4 to 5 minutes in water at 47° to 48° C. proved the most effective treatments. Five pods were set from 79 treated, but unpollinated, flowers of *Indigofera hirsuta* L., indicating that the pollen was effectively inactivated by the treatment in a large majority of the cases. The set of 42 pods from 72 treated flowers, after hand pollinations with untreated pollen from adjacent flowers, indicates that most of the female structures were still functional. Considerable

variation in the effectiveness of hot-water treatment was found from plant to plant and from day to day, however, and it appears that complete and consistent emasculation, using the hot-water technique, is unlikely. Nevertheless, the technique appears to offer some promise to the breeder working with this difficult genus.

LEGUME INOCULATION. R. H. Freyre.

In order to determine if the early growth rate of tropical kudzu (*Pueraria phaseoloides* (Roxb.) Benth.) could be accelerated, tests were run in which seeds were inoculated with different cultures of nitrogen-fixing bacteria. Cultures used were (1) a special inoculum prepared by L. W. Erdman of BPISAE, (2) a commercial inoculum supplied by the Nitragin Co., Milwaukee, Wis., and (3) a soil paste prepared from a field which had grown kudzu for several years. In addition, a control treatment was used in which no inoculation was made. The tests were run at three locations (south field, Las Mesas, and Anasco), four replications in Latin square design were used, and plants were harvested and weighed at three different ages. The experiment was run twice—once during the dry season and once during the rainy season. Although the Erdman and Nitragin treatments slightly outyielded the mud and control treatments, no consistent significant differences in yields were observed. This would seem to indicate that efficient strains of nitrogen-fixing bacteria are already present in these soils and that commercial inoculation cannot be expected to improve the early growth of tropical kudzu in the Mayaguez area.

LEGUME TOXICITY. H. E. Warmke and R. H. Freyre.

It was reported last year that guinea pigs fed on forage of *Indigofera endecaphylla* Jacq. and *I. mucronata* Spreng. ex DC. aborted all or most of their young, usually at an early stage. With the thought that such abortion might possibly be caused by a vitamin or protein deficiency, the diets of eight pairs of animals were supplemented with approximately 5 grams per animal per day of a mixture consisting of equal parts of powdered dried brewer's yeast, crude casein, and wheat germ meal. Animals fed on this supplemented ration for nearly 6 months failed to show an improvement in reproductive behavior. This indicates that the abortions were not caused by a dietary deficiency, but probably by a toxic substance in the legumes themselves. After 15 months on *Indigofera* forage, the guinea pigs were returned to control rations (tropical kudzu). Within 4 months all females had borne litters of live young and reproduction is continuing normal. These results would indicate that *I. endecaphylla* and *I. mucronata*, although markedly lowering reproductive efficiency in guinea pigs, apparently do not cause permanent damage to the reproductive system.

TOXIC COMPONENTS OF TRAILING INDIGO. M. P. Morris and C. Pagán.

Several fractionating procedures that are frequently used in the study of natural extracts were carried out in an attempt to isolate the toxic components of trailing indigo. The products of each step were bioassayed for toxicity in order to follow the toxic materials through the scheme. The results led to the following simple method for isolating the toxic components of trailing indigo: (1) Extract with water; (2) adsorb toxic components on charcoal after clarifying with lead acetate; (3) extract toxic components from charcoal with large

volumes of ether; and (4) evaporate ether to obtain toxic components.

The above procedure yielded a thick, water-soluble, pungent oil which was highly toxic to chicks. This oil was further separated by molecular distillation into three fractions: (1) Low boiling, (2) high boiling, and (3) residue. All three fractions were highly toxic to chicks. The high-boiling fraction crystallized upon cooling into colorless plates, and was the most toxic fraction. Approximately 5-milligram doses in 0.5 ml. of water caused complete paralysis in a few minutes and death within an hour when given orally to chicks weighing 65 to 75 grams. When given in smaller doses the symptoms were identical with those obtained by feeding a 20-percent trailing indigo ration for 4 days.

Effect of Trailing Indigo on Chicks. C. Pagán, M. P. Morris, and H. E. Warmke.

During a series of feeding tests to determine the toxic dose of trailing indigo for baby chicks, the change of weight of the chicks was first considered as a criterion of toxicity. However, it soon became evident that the rate of growth of the chicks was associated with reduced food consumption. This factor, which is often overlooked in toxicity studies, was therefore given careful consideration in the establishment of the toxic doses.

The ground legume was mixed with starting mash at concentrations of 5, 10, 15, and 20 percent by weight. The control ration consisted of the starting mash. These rations were fed to groups of 10 New Hampshire chicks, 5 days old. The chicks were kept on control and experimental diets for 4 days, during which time those on the 10, 15, and 20 percent trailing indigo rations developed the characteristic toxicity syndrome. On the fourth day these chicks were returned to the normal ration of starting mash. Chicks on 5 percent trailing indigo ration were unaffected after 4 days of treatment and were kept on this ration for an additional 5 days. At this time they had consumed 21 percent less food and had gained 37 percent less weight than the controls. The test animals were weighed daily and food consumption recorded.

The change in weight of chicks was directly related to both the total food consumed and to the concentration of trailing indigo in the rations. However, it should not be concluded that the change in weight is due to the toxic factor of trailing indigo, because the same changes in weight are also produced by starvation rations. To reach such a conclusion it must first be demonstrated that the toxic factor is the cause of the reduced-food consumption. Feeding experiments have shown that guinea pigs often refuse to eat trailing indigo rations and even tropical kudzu (when not accustomed to these legumes), and starve to death. This would suggest that loss in weight of test animals fed trailing indigo might be caused by a reduced feed consumption due to unfamiliarity with this ration or its low palatability rather than to a true toxic action.

Toxic Components in Trailing Indigo. C. Pagán and M. P. Morris.

The hydrogen cyanide content of trailing indigo was found to be approximately 30 p.p.m. This was more than 10 times the amount found in 10 other legumes analyzed at the same time. Cyanogenetic glucosides were, therefore, considered as the possible cause of the

toxicity of trailing indigo. If cyanogenetic glucosides were the cause of toxicity, then the hydrogen cyanide isolated from one toxic dose of trailing indigo should produce symptoms when administered orally to the test animal.

The toxic dose of trailing indigo was established at 8 grams of dry plant material for a 100-gram chick. At 30 p. p. m. this toxic dose would contain the equivalent of 0.24 mg. of hydrogen cyanide. When the amount was administered orally in the form of sodium cyanide no ill effects were observed. The daily administration of this amount for 5 consecutive days produced no ill effects. Further, when the daily dose was increased three times and given daily for 7 days, no ill effects were noted. In the latter case, the test animals had consumed 22 times the amount of hydrogen cyanide found in one toxic dose of trailing indigo. This indicates conclusively that the toxicity of trailing indigo was not due to hydrogen cyanide produced by the hydrolysis of cyanogenetic glycosides.

Alkaloids were at one time considered as the possible cause of the toxicity of trailing indigo. These toxic materials were considered because of the symptoms that developed in the test animal, and because of the fact that test animals always recovered rapidly and completely when removed from rations containing trailing indigo. It is now known that alkaloids present could not be the cause of the toxicity. This conclusion is based on the fact that the alkaloid fraction from 100 grams of trailing indigo produces no ill effects when administered to 100-gram chicks. The same chicks were severely affected after the consumption of 8 grams of trailing indigo.

Method for Determining Toxic Component in Trailing Indigo. C. Pagán and M. P. Morris.

Columnar and paper chromatography has proved particularly effective for the resolution of organic mixtures and for the isolation and identification of the components.

In using this technique for assay of trailing indigo extracts, an aliquot representing 1 gram of dry material was evaporated to a volume of about 0.1 ml. This solution was applied, a few microliters at a time, to a point on a strip of Whatman No. 4 filter paper, 1½ inches from the lower edge. This point was maintained at 100° C., during the application of the solution. In this way the spot was kept to a minimum size while the full amount of 0.1 ml. of extract was added. One milligram of the pure toxic compound was then placed in a similar manner at an adjacent point to serve as a standard. The strip of paper was supported in a closed vessel so that the lower edge of the paper dipped into butanol saturated with water. Formation of the chromatogram required 16 to 20 hours, during which time the toxic mixture from trailing indigo was resolved distinctly into three acids. The acids were colorless and their position on the paper unknown until the sheet was sprayed with bromophenol blue solution. This indicator turns from blue to yellow in the presence of acids. Thus, the positions of the acids on the paper were indicated by three yellow spots. The quantity of toxic acid in the aliquot was determined by comparing its chromatogram with that of the standard.

The method described above was applied to samples of sun-grown and shade-grown *Indigofera endecaphylla* (trailing indigo) and to *I. mucronata*. The resulting chromatograms showed that the three

extracts contained the same organic acids and that *I. mucronata* contained the least amount of toxic material. These analyses confirm the results of feeding trials carried out at this station. The chromatograms also show that the concentrations of the toxic component in the sun-grown and shade-grown samples of *I. endecaphylla* are approximately the same.

In another trial a sample of kudzu, *Pueraria phaseoloides*, was extracted and chromatographed by identical procedures. The chromatogram that developed was entirely different and showed none of the toxic acid which is present in trailing indigo and *I. mucronata*. This is in agreement with feeding tests.

The method of analysis reported here should prove a simple and useful method for eliminating toxic strains of Indigofera and other legumes in the forage-breeding program.

IDENTIFICATION OF TOXIC PRINCIPLE OF TRAILING INDIGO. M. P. Morris and C. Pagán.

A series of extractions with various solvents showed that the toxic substance could not be extracted by long periods (24 hours) with nonpolar solvents such as chloroform, benzene, and carbon tetrachloride or with ether. It was found that the toxic material was readily extracted with hot water (80° to 100° C.) in 3 hours. Further, it was found that the toxic material could be extracted from this aqueous solution by repeated extractions with ether. This indicated that the toxic material must exist in the plant material as the aglycone of some presently unknown glycoside, since it was originally insoluble in ether.

The toxic material is readily obtained in crystalline form from ether or benzene solutions by the addition of petroleum ether. The crude crystalline material melts at 63° to 65° C., and a highly purified sample obtained from a silicic acid column melts at 67.5° to 68° C. Qualitative tests show the presence of carbon, hydrogen, and nitrogen. Quantitative analysis of carbon, hydrogen, and nitrogen indicate an empirical formula of $C_3H_5NO_4$. Aqueous solutions are strongly acidic and points of inflection on the titrimetric curve indicate equivalent weights of 58 and 118. The molecular formula of this compound must therefore be $C_3H_5NO_4$ (119.08). Molecular weight determinations in benzene gave figures of 235 to 245, showing that this strong acid dimerizes in nonpolar solvents. Since the acid was dibasic, it seemed probable that the 4 oxygen atoms in the molecule could be accounted for by 2 carboxyl groups. The fact that this compound smoothly evolved carbon dioxide when heated to 130° to 140° C. added weight to this idea. However, qualitative tests showed that the nitrogen atom was in an oxidized state, and therefore the toxic compound could not be a dicarboxylic acid.

With the above information, a literature search soon showed the toxic acid obtained from *Indigofera endecaphylla* is identical with hiptagenic acid, the aglycone of hiptagin, a toxic glycoside first isolated by Gorter [4] from *Hiptage benghalensis* (L.) Kurz (madablota), a plant native to India. This conclusion was not obvious from a

[4] GORTER, K. L'HIPTAGINE, GLUCOSIDE NOUVEAU RETIRÉ DE L'HIPTAGE MADABLOTA GAERTN. Buitenzorg, Java. 'S Lands Plantentuin. Bul. Jard. Bot. Buitenzorg (ser. 3) 2: 187–202. 1920.

comparison of the physical and chemical properties of the two compounds. Fortunately, however, three plants of *H. benghalensis* had been introduced from the Orient (P. I. 116513) by the Bureau of Plant Industry in 1939 and were being maintained here at this Station. This permitted a direct comparison of the two compounds. Both compounds gave the same crystalline forms and both melted at 67.5° to 68° C., and have the same composition, and identical titrimetric curves. Further, and more conclusive, is the fact that when mixed in equal quantities, the mixture obtained melts at 67° to 68° C.

In Gorter's original paper he attempted to explain the unusual and often complex chemical reactions of hiptagenic acid by suggesting the formula $HONH-\overset{\overset{O}{\|}}{C}-\overset{\overset{OH}{|}}{C}=CHOH$ for hiptagenic acid. In 1943 Carter [5] reported the isolation of hiptagenic acid from *Cornyocarpus laevigata* Forst. Carter suggested the formula $HON\text{-}CH-\underset{\underset{OH}{|}}{CH}-COOH$ for the acid.

At the time of the above suggestions it was generally believed that organic nitrocompounds did not exist as such in natural products. This belief was based on the fact that all attempts to isolate nitrocompounds from natural products had been unsuccessful. For this reason, undoubtedly, formulas containing nitrogroups were not considered. However, since that time a nitrocompound (Chloramphenicol) has been isolated from natural sources.[6] This discovery of naturally occurring nitrocompounds compelled Carter [5] to reconsider his suggested formula for hiptagenic acid. As a result he soon showed [7] that hiptagenic acid was identical with beta nitropropionic acid. He did this by synthesizing beta nitropropionic acid and showing that it was identical with the acid obtained from *Cornyocarpus laevigata*. Recently another group of investigators [8] has shown that beta nitropropionic acid is a metabolic product of *Aspergillus flavus*.

A comparison of the chemical and physical properties of the four above-mentioned compounds, all of which melt at 67° to 68° C., shows that they are more than likely identical. It appears, with almost certainty, that this toxic compound is beta nitropropionic acid. Nevertheless, the present investigators have observed one reaction which throws doubt on the conclusion that hiptagenic acid is identical with beta nitropropionic acid.

The nitrogen of hiptagenic is liberated quantitatively by 20 percent sodium hydroxide solution. Although nitrocompounds rearrange in basic solution and often give ammonia as one of the products, quantitative conversion would be unusual for a nitrocompound. This point is currently being checked. Beta nitropropionic acid is being pre-

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[5] CARTER, C. L. KARAKIN, THE GLUCOSIDE OF CORNYOCARPUS LAEVIGATA, AND HIPTAGENIC ACID. Jour. Soc. Chem. Ind. 62: 238–240, illus. 1943.

[6] EHRLICH, J., and others. CHLOROMYCETIN, A NEW ANTIBIOTIC FROM A SOIL ACTINOMYCETE. Science 106: 417. 1947.

[7] CARTER, C. L., and MCCHESNEY, W. J. HIPTAGENIC ACID IDENTIFIED AS β-NITROPROPIONIC ACID. Nature 164: 575–576. 1949.

[8] BUSH, M. T., TOUSTER, O., and BROCKMAN, J. E. THE PRODUCTION OF β-NITROPROPIONIC ACID BY A STRAIN OF ASPERGILLUS FLAVUS. Jour. Biol. Chem. 188: 685–693, illus. 1951.

pared, and will be compared directly with hiptagenic acid isolated from *Hiptage benghalensis*. A direct comparison was not made by the previous investigators.

WHEAT RUST INVESTIGATIONS

SELECTIONS OF RUST RESISTANT WHEAT. **T. Theis.**

A cooperative program to determine sources of wheat resistant to stem rust was established with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. D. A. Wheat nurseries were established at St. Croix, V. I., Obregon and Chapingo, Mexico, and in the greenhouse at the Plant Industry Station, Beltsville, Md.[9]

The Caribbean area was selected for a nursery because wheat is not grown as a crop and there is a freedom from wheat rust, and because the high relative humidity that occurs nightly and the heavy dew deposits during the winter season facilitates the establishment of the disease by inoculation. Once the rust is established, it is quite certain that only those races that were introduced will be present in the field. There is little danger of windborne contamination with other races.

Only one race of rust was used in the St. Croix plots and in the greenhouse tests at Beltsville. The race selected was 15B. It is one that has increased in importance during recent years and has caused extensive losses in the wheat areas of the United States. Inoculations were made with a composite of biotypes of 15B obtained from Dr. E. C. Stakman. These were originally isolated from collections of the types prevalent in commercial fields in the United States during 1950 and 1951. The Mexican nurseries were dependent on natural infection for the test.

The wheat nursery on St. Croix, V. I., was established on land provided by the former Agricultural Experiment Station. The nursery was composed of 4,876 wheat varieties obtained from plant breeders of the U. S. Department of Agriculture, State experiment stations, and experiment stations in Canada and Mexico. To these were added foreign introductions that were selected from among the 13,500 wheats in the U. S. D. A. world collection.

There was considerable variability in the stage of maturity of the wheats grown on St. Croix when the rust notes were taken, ranging from rosette to heading stage. In general, all of the spring wheats headed, and the true winter wheats remained in the rosette stage. A considerable number were intermediate in that they had culm elongation but had not headed when the notes were taken. Undoubtedly the severity readings on the majority of these would have been higher if the rust had had more time to develop. A leaf reading was taken on those entries which remained in the rosette stage.

There was adequate resistance from several sources to the rust races used in these tests. Of 35 introductions from Kenya 9 were outstanding for resistance at St. Croix, in Mexico, and in the seedling

[9] C. V. Lowther, T. Theis, and H. A. Rodenhiser, were responsible for the St. Croix experiments. The greenhouse tests at Beltsville were carried out by the late C. V. Lowther. The experiments in Mexico were conducted by N. E. Borlaug and B. B. Bayles.

tests at Beltsville, Md. They are as follows: Kenya 58, K. 117A, K. 291 J. I. I. I., K. 294B. 2. A. 3, K. 321 BT. 1. B. 1, K. 338 AA. 1. A. 2, K 338 AC. 2. E. 2, K. 350 AD. 9. C., and K. 351 A. S. 1. B. 2. Hybrids of some of these Kenyas with one of several commercial wheats were likewise highly resistant, for example, selections from K. 58 or K. 117A × Newthatch, Mida, or Pilot.

Egypt Na 101 has been susceptible in some tests in Mexico; however, it has been resistant in South America and to the 15B collection used at St. Croix and in the Beltsville tests. Certain hybrids involving Egypt Na 101 have been particularly outstanding for resistance, namely crosses with Timstein supplied by Dr. Borlaug.

A few selections from crosses of Frontana with Newthatch were resistant. Entries 461, 462, and 464 (Newthatch × Frontana) were resistant in these tests as they had been in South American trials. Several lines, however, from crosses of Frontana with Thatcher, or Newthatch that were resistant in South American countries were susceptible at St. Croix.

Out of the approximately 4,000 foreign plant introductions tested, only 31 entries had an appreciable degree of resistance in all tests. These are not all new sources of resistance, however, since some represent the Kenya and Egypt types previously reported.

There appears to be several sources of resistance in the durum wheats. On the basis of these and previous tests the following durums are most promising: Beladi 116 from Egypt (P. I. 153777); Tremez Molle, Tremez Preto, and Tremez Rijo from Portugal; and Chapinge from Canada. Trigo Glutinoso (P. I. 174699) was resistant in the present tests but had 25 percent infection in Peru.

Sixty-four of Mr. W. J. Sando's (Triticum × Agropyron) × Triticum crosses were grown in the St. Croix nursery. Ten of these were free from rust but each had not advanced beyond the rosette stage at the time the notes were taken. Both wheatlike and agropyronlike individuals are represented in the resistant lines.

The 15B inoculum used to establish infection in the nursery at St. Croix and that used in the greenhouse at Beltsville came from a common source. In many cases, however, the readings obtained differ markedly. For example, Kenya Standard was highly resistant in the greenhouse, but developed 80 percent rust at St. Croix. Sapporo haur Komugi ichigo is a similar case. In every case where this difference occurred in the adult-plant readings, the more susceptible reaction was at St. Croix. In all probability these differences are due to environmental conditions.

WEATHER

Rainfall was below the average for the fiscal year ended June 30, 1953. The rainfall for the last 6 months of 1952 was 35.11 inches, or 14.65 inches below the 54-year average of 49.76. For the first 6 months of 1953 a precipitation of 32.67 inches was recorded, which was 3.24 inches above the 55-year average of 29.43. The total rainfall for the fiscal year was 67.88 inches, or 11.41 inches below the 54-year average of 79.19 inches.

The mean temperature recorded at Mayaguez, P. R., for the fiscal

year ended June 30, 1953, was 77.15° F., which was 0.07° F. below the 53-year average of 77.22° F.

TABLE 1.—*Weather conditions at the Federal Experimental Station, Mayaguez, P. R., during the fiscal year 1952–53.*

Month	Precipitation [1]			Temperature [2]				
	Total	Greatest in 24 hours	Days with 0.01 inch or more	Mean maximum	Mean minimum	Mean	Maximum	Minimum
1952	*Inches*	*Inches*	*Inches*	*° F.*	*° F.*	*° F.*	*° F.*	*° F.*
July	8. 21	2. 20	19	90. 3	69. 5	79. 9	94	67
August	9. 55	1. 63	19	90. 9	69. 7	80. 3	94	68
September	10. 95	2. 70	24	89. 8	69. 8	79. 8	94	69
October	2. 33	. 43	17	90. 1	69. 5	79. 8	94	67
November	3. 59	1. 10	14	86. 6	68. 0	77. 3	90	65
December	. 48	. 43	2	84. 7	64. 3	74. 5	89	60
1953								
January	1. 38	. 88	7	85. 3	63. 4	74. 4	90	60
February	. 36	-------	-------	84. 4	63. 2	73. 8	88	60
March	3. 36	1. 80	7	86. 4	63. 4	74. 9	92	59
April	6. 48	1. 24	16	88. 1	64. 0	76. 1	93	61
May	10. 46	1. 92	19	87. 6	65. 5	76. 6	91	60
June	10. 63	1. 65	19	89. 4	67. 3	78. 4	93	65

[1] 54-year average: July, 10.47 inches; August, 10.91 inches; September, 10.86 inches; October, 9.07 inches; November, 5.87 inches; December, 2.58 inches.

55-year average: January, 1.98 inches; February, 1.94 inches; March, 3.57; April, 4.96 inches; May, 8.23; June, 8.75.

[2] 53-year average: July, 79.1°; August, 79.4°; September, 79.5°; October, 79.2°; November, 77.6°; December, 75.9°.

54-year average: January, 74.6°; February, 74.7°; March, 74.9°; April, 76.2°; May, 77.8°; June, 77.8°.

PUBLICATIONS ISSUED

In addition to the annual report of the Federal station for the fiscal year 1952, the following publications of the Department were issued during the year:

LOWTHER, C. V., THEIS, T., and RODENHISER, H. A. Summary report on the reaction of wheat to stem rust at St. Croix, Virgin Islands; Mexico, and Beltsville, Maryland in 1951–52 U. S. Dept. Agr., Bur. Plant Indus., Soils, and Agr. Engin., 56 pp. 1952. [Processed.]

WINTERS, H. F. Some large-leaved ornamental plants for the Tropics. Puerto Rico (Mayaguez) Fed. Expt. Sta. Cir. 35, 92 pp., illus. 1952.

The following articles were published by the station staff in periodicals outside the Department:

HAGEMAN, R. H., PAGAN, C., and LOUSTALOT, A. J. The effect of elevation on growth, carbohydrates and insecticidal constituents of derris and lonchocarpus. Turrialba 2 (4): 148–152. 1952. (Abs.) Trop. Agr. [Trinidad] 30: 42. 1953.

LOUSTALOT, A. J., and MUZIK, T. J. The effect of 2,4-D on apparent photosynthesis and developmental morphology of velvet beans. *In* South. Weed Conf. Proc., New Orleans, La., p. 57. Feb. 11–13, 1953. (Abs.) Amer. Soc. Hort. Sci. Proc. 61: 324. 1953.

MORRIS, M. P., and PAGÁN, C. The isolation of the toxic principles of mamey. Amer. Chem. Soc. Jour. 75: 1489. 1953.
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MUZIK, T. J. Citrus in West Africa, with special reference to Liberia. Econ. Bot. 6: 246–251. 1952.
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THEIS, T. An undescribed species of ergot on *Panieum maximum* Jacq. var. Common Guinea. Mycologia 44: 789–794, illus. 1952.
WARMKE, H. E. Studies on natural pollination of *Hevea brasiliensis* in Brazil. Science 116: 474–475. 1952.
WARMKE, H. E., FREYRE, R. H., and GARCÍA, J. Evaluation of some tropical grass-legume associations. Trop. Agr. [Trinidad] 29: 115–121, illus. 1952.
WARMKE, H. E., FREYRE, R. H., and MORRIS, M. P. Studies on palatability of some tropical legumes. Agron. Jour. 44: 517–520. 1952.
WINTERS, H. F., and LOUSTALOT, A. J. The effect of light and nitrogen levels on growth and alkaloid content of young *Cinchona ledgeriana*. Plant Physiol. 27: 575–582, illus. 1952.
WINTERS, H. F., and RODRIGUEZ-COLON, R. Storage of mangosteen seed. Amer. Soc. Hort. Sci. Proc. 61: 304–306. 1953.

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